GW25-e0091

Effects of Cardiac Stem Cells Transplantation on the Ventricular Fibrillation Threshold in Rats with Myocardial Infarction in Short-Term, Medium-Term and Long-Term Period

Zheng Shaoxin[1], Wang Tong[1], Chen Jian[2], Liang Peifen[1], Fang Yanling[1], Huang Hui[1], Wu Wei[2], Wang Jingfeng[1]

[1]The Sun Yat-sen Memorial Hospital of Sun Yat-sen University, Guangzhou, China, [2]The Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, China

Objectives: Arrhythmia is of concern after cardiac stem cells (CSCs) transplantation in repairing infarcted myocardium. However, whether transplanted CSCs improved ventricular fibrillation threshold (VFT) in the myocardial infarction model is still unclear. We sought to investigate the VFT in rats with myocardial infarction treated with cardiac stem cells.
Methods: Myocardial infarction was induced by ligation of the left anterior descending artery in 60 rats. 2 weeks later, animals were randomized to receive of 5×10^6 CSCs labeled with PKH26 in phosphate buffer solution (PBS) (30 rats) or PBS (30 rats) alone injection into the infarction zone in the anterior ventricular free wall. 2, 6 and 12 weeks after CSCs or PBS injection, VFT was measured on infarct zone, infarct marginal zone and non-infarct zone (each period for 10 rats in CSCs group and 10 rats in PBS group). Labeled CSCs were observed in 5 μm cryostat sections from each harvested heart.
Results: In the CSCs group, there were significant improvements in VFT on the infarct zone, infarct marginal zone and non-infarct zone compared with the PBS groups in short-term (infarct zone: 10.2±2.4mA vs 3.7±0.9mA, infarct marginal zone: 8.9±1.9mA vs 3.6±1.6mA, non-infarct zone: 7.8±2.2mA vs 2.3±0.7mA. P<0.05) and medium-term period (infarct zone: 8.4±3.0mA vs 4.0±1.6mA, infarct marginal zone: 6.4±2.5mA vs 2.5±0.8mA, non-infarct zone: 7.0±2.6mA vs 3.4±1.0mA. P<0.05). However, in long-term period, VFT was improved only on non-infarct zone (6.5±2.0mA vs 3.5±1.4mA. P<0.05). Labeled CSCs were identified on infarct zone and infarct marginal zone, and expressed Connexin-43 and α-sarcomeric actin.
Conclusions: The CSCs transplantation can improve the VFT in rats with myocardial infarction in the short-term and medium-term period. However, as time goes on, the improvement may be attenuate. The CSCs can differentiate into cardiomyocytes on infarct zone and infarct marginal zone.

GW25-e0559

CD51-Positive Cells from Mouse Myocardial Tissue: Isolation and Characterization

Peng Chao-Quan, Chen Yang

Department of Cardiology, The Third Affiliated Hospital, Sun Yat-Sen University

Objectives: The objectives of this study were to determine the distribution of CD51-positive ($CD51^+$) cells in normal heart tissue in C57 mice, to culture and expand them in vitro, and to verify their proliferation and differentiation potential. We thus hoped to provide experimental evidence of CD51 as a marker of cardiac-derivedstem cells.
Methods: We used RT-PCR and Q-PCR to observe the expression levels of CD51+ cells in neonatal 1-day-(1d), 7-day-(7d), 1-month-(1m), and 3-month-old (3m) C57 mouse hearts. The distribution of CD51+ cells in 1d and 3m C57 mouse heart tissue was detected by immunofluorescence staining. The proliferation and multi-differentiation capacity of CD51+ cells were tested by CCK-8 analysis and osteogenic and adipogenic induction analyses, respectively. Finally, we tested the differentiation potential of cardiomyocytes and smooth muscle cells by induction experiments after cell passage to P6 and briefly analyzed the induction efficiency.
Results: Expression levels of $CD51^+$ cells were almost identical among mice of the same age, and the levels declined with age. $CD51^+$ cells were widely expressed in the atria and ventricles of 7d C57 mice. We also found that the expression levels decreased markedly in 3m C57 mouse heart tissue; only a few $CD51^+$ cells remained in the same locations in the atria and ventricles. Flow cytometry showed that the positive rate was approximately 4.6% in 7d C57 mice, suggesting sufficient quantities of cells for vitro culture. We also obtained $CD51^+$ cells by FACS from the hearts of 7d C57 mice and found that these cells could proliferate with adherence status in serum-free medium. Their morphology was similar to that of bone marrow MSCs. We proved that besides differentiating into adipocytes and osteoblasts, these cells could also differentiate into smooth muscle cells and cardiomyocytes in vitro after cell passage yielded stable cell lines. Compared with bone marrow-derived MSCs, these cells were found to exhibit low efficiency of differentiation into adipogenic and osteogenic lines but high efficiency of differentiation into cardiomyocytes and smooth muscle cells (about 10-15% respectively). On testing proliferation ability when the cells extended to P5, P15, and P25, we found that the proliferation capacity had no obvious difference, and proliferation continued.
Conclusions: $CD51^+$ cells in murine myocardial tissue are cell subsets with stem cell characteristics similar to those of MSC-like stem cells in the heart. Their proliferation and differentiation potentials are similar to those of traditional bone marrow MSCs, but they also possess their own biological characteristics. These differences can be attributed to the original microenvironment of the heart. Thus, cardiac-derived MSC-like stem cells might possess repair mechanisms different from those of MSCs derived from bone marrow or other tissues. Furthermore, these cells may be more beneficial in the treatment heart disease in particular. The underlying mechanisms are not clear, warranting further research to provide a new theoretical basis for exploring effective stem cell therapy for heart disease.

GW25-e0600

Chronic angiotensin-(1-7) administration improves endothelium-dependent vasodilation in rabbits with balloon injury

Wutao Zeng[1], Weiyan Chen[2], Xiuting S[1], Xiang Wang[1], Meiling Liang[1], Zhenxun Li[1]

[1]Division of cardiology, Cardiovascular Medical Department, the First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, [2]Department of Intensive Care Unit, the Second Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

Objectives: Endothium-denpendent vasodilation is impaired in vascular restenosis, even before the development of atherosclerosis. The purpose of this study was to determine whether infusion of angiotensin-(1-7), a biological active member of the renin-angiotensin pepetide family, improves endothelium-dependent vasodilation in rabbits with balloon injury.
Methods: Tweenty-four New Zealand white rabbits underwent sham surgery or angioplasty in abdominal aorta. The animals were divided into 3 groups, which were sham, control, ANG-(1-7). Subsequently, an osmotic minipump was implanted to deliver saline, ANG-(1-7) ($576\mu g\cdot kg^{-1}\cdot d^{-1}$) for 4 weeks. After 4 weeks, abdominal angiography was done through the left femoral, and then the diameter of the lumen was calculated. After angiography, rabbits were killed for histological analysis, immunohistochemistry analysis of Protein TGF-β1 and westernblot analysis of protein Smad2.
Results: All rabbits with vascular injury showed that the abdominal aortic flow was significantly increased after balloon dilation, and decreased 4 weeks after restenosis. After vascular injury for 4 weeks, control group has significantly decreased luman diameter than sham group [(2.88±0.08) mm vs (3.85±0.03) mm, P<0.05]. ANG-(1-7) group has significantly increased luman diameter than control group [(4.11±0.10) mm vs (2.88±0.08) mm, P<0.05]. Compared to control group, ANG-(1-7) group neointima significantly decreased thickness, neointimal area and restenosis rate [(207.51±16.70) μmvs (448.08±15.30) μm, P<0.05; (2.66±0.09) mm^2vs (4.08±0.02) mm^2, P<0.05; (28.13±2.74) % vs (40.13±2.74) %, P=0.008, respectively]. But no difference was found between sham group and ANG-(1-7) group. The expressive level of TGF-β1 in control group was significantly higher than ANG-(1-7) group (2.37±0.03 vs 1.46±0.02, P<0.05). The expressive level of TGF-β1 in injury vessel was positively correlated with the restenosis rate (r=0.73, P<0.001). The expressive levels of Smad2 were 6.96-fold and 2.69-fold in control group and ANG-(1-7) -treated group compared to sham group 4 weeks after balloon injury.
Conclusions: ANG-(1-7) attenuates vascular restenosis and improves endothelium-dependent vasodilation in rabbits with balloon injury. The mechanism was associated with TGF-β_1/Smad Signaling Pathway.

GW25-e0735

Role of Wnt4/Dvl-1/ß-Catenin Signal Pathway in Balloon-Injured Carotid Artery Restenosis

Hua Junyi[1], Xu Yun[2], Jiang Xuhong[1], He Yuzhou[1], Ye Wu[1], Pan Zhimin[1]

[1]The First Affiliated Hospital, Zhejiang University of Traditional Chinese Medicine, [2]Medicine Communication and Management Center of ZheJiang Province

Objectives: To evaluate whether Wnt4/Dvl-1/ß-catenin signalling pathway modulates balloon-injuried artery restenosis and try to find potential therapeutic gene target of anti-vascular restenosis.
Methods: Cell experiment in vitro: Cultured vascular smooth muscle cells (VSMC) were stimulated by AngII (10^{-6}mol/L). SiRNA-dvl-1 was employed to silence gene of dvl-1. Atrovastatin of different concentration (0.1μmol/L, 1μmol/L, 10μmol/L) were also enrolled in the cultured system. The SMCs proliferation was determined by MTT assay. Several Wnt genes and proteins such as Wnt4, dvl-1, ß-catenin were quantitated separatedly by Real-Time quantitative-PCR and Western blot assay. Animal experiment in vivo: Rat model of balloon-injured carotid artery was established. SD rats were randomized into 3 groups: sham operation group, operation group, atrovastatin group. Ligated carotid arteries were removed 6 weeks after balloon injuried and to assess intimal lesion size by HE staining. Immunohistochemistry was performed on rat carotid artery to test Wnt4, dvl-1, ß-catenin.
Results: In vitro: (1) VSMC proliferation induced by AngII could be inhibited by atrovastatin in a concentrated-dependent manner and siRNA-Dvl-1 (P<0.05). (2) High expression of wnt signals (wnt4, dvl-1, ß-catenin) mRNA and proteins induced by AngII could be down-regulated significantly by atrovastatin with certain concentration that inhibit SMC proliferation (P<0.05). (3) High expression of collegan I, collegan III's mRNA and proteins could be down-regulated significantly by atorvastatin and siRNA-Dvl-1 (P<0.05). In vivo, compared with model group, (1) hyperplasia of artery intima, area of neointimal, thickness of intima and area ratio of intima/ tunica media in atrovastatin group were all reduced (P<0.05); (2) immunohistochemistry and western blot showed expression of wnt signals as Wnt4, dvl-1 and ß -catenin in injured artery of atrovastatin group were reduced (P<0.05); (3) collagen volume fraction by collagen specific staining with Picric Sirius red and proteins expression of col1, 3 in injured artery by western blot reduced significantly in atrovastatin group (P<0.05).
Conclusions: (1) wnt4/dvl-1/ß-catenin signalling pathway modulates proliferation of smooth muscle cells from rats aorta induced by AngII; (2) Atrovastatin could inhibit balloon-injured carotid artery restenosis by down-regulating wnt signalling pathway. Wnt signalling pathway may become a novel potential therapeutic gene target of anti-vascular restenosis.